spike-evoking epochs. The effects of interactions between spikes is minimized by selecting signals that generate spikes with relatively long interspike intervals. We study two distinct cases characterized by either a smooth or discontinuous dependence of frequency on the applied current. In the setting of noise-induced spiking the frequency content of the input signal has little impact on STR. Rather, increased STR is observed for a certain increase in the average amount of current delivered during a fixed time interval in combination with a favorable time profile. These computational results are complemented by an analytical approximation for the density of the phase difference of two coupled stochastically forced oscillators, considering different relative sizes of the intrinsic and extrinsic noises. When common noise forcing dominates, there is a stronger probability of synchronization related to STR. In contrast, if the intrinsic noises are not of identical strength, there is a synchronized lag between oscillations.

1173-Symp
Deterministic Versus Stochastic Variability in the Mammalian Cell Cycle
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Cell-to cell variability in cell cycle duration was observed long ago however its sources are still unknown. A surprising feature of cell cycle duration inheritance is that it seems to be lost within one generation but to reappear in the next generation, generating poor correlation between mother and daughter cells but high correlation between cousin cells. This observation suggests the existence of an underlying deterministic process. We developed an experimental system that precisely measures the cell cycle duration of thousands of mammalian cells along several generations and a mathematical framework that allows discrimination between stochastic and deterministic processes. In contrast to previous understanding, we show that the inheritance of the cell-cycle duration follows a deterministic process. We build a deterministic chaos toy model for cell-cycle inheritance that reproduces the main features of our data. Our approach constitutes a general way to distinguish between stochastic and deterministic processes in lineages of cells or organisms, and may help predict and, eventually, control cell-to-cell heterogeneity in various systems, such as cancer cells under treatment.

Symposium: Regulation of Cytoskeletal Motors

1174-Symp
Mechanistic Insights of Dynein Motor Action from Electron Microscopy Studies
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Dynein motors are the largest of the cytoskeletal motor proteins. They perform critical functions in eukaryotic cells, carrying cargoes along microtubule tracks and exerting pulling forces on microtubules in a variety of cellular processes. Dynein motors use ATP hydrolysis to perform these functions by moving towards the minus ends of microtubules. A major goal of our research is to understand the structure and conformational changes that allow dynein, a member of the AAA^+ superfamily of ring-shaped ATPases, to perform these functions.
We have used cryo-electron microscopy and single-particle image processing to determine (ab initio) three-dimensional structures of a native (full-length) flagellar dynein (dynein-c from the single-celled alga Chlamydomonas) and of an engineered cytoplasmic dynein motor domain (from the slime mold Dictyostelium) lacking the cargo-binding tail domain, in different nucleotide states. The structures show key sites of conformational change within the AAA^+ ring and a large rearrangement of the ''linker'' domain, involving a hinge near its middle.
Analysis of a mutant in which the linker ''undocks'' from the ring indicates that linker remodeling requires energy that is supplied by interactions with the AAA^+ modules. We find flexing of the tail domain of dynein-c and the stalks of both dynein isoforms, relative to the AAA^+ ring in the frozen-hydrated state. Fitting the dynein-c structures into three-dimensional cryo-tomograms of Chlamydomonas flagella suggests how the mechanism of linker remodeling could drive sliding between microtubules. This also has implications for the processive stepping of cytoplasmic dynein dimers undergoing cargo transport and force exertion.
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1175-Symp
Reconstitution of Dynamic Axonemal Complexity with using a Bottom up Strategy
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The complexity of the flagellar axoneme derives from ca. 600 types of modular building block assembled hierarchically. Among these building block, axonemal dyneins are indispensable for flagellar motility. On each of nine doublet microtubules cyclically arrayed in an axoneme, dyneins are aligned in two rows, outer- and inner-arm dyneins. In Chlamydomonas, the model organism for flagellar motility, several major subspecies of dyneins have been described; one outer-arm dynein with three different heavy chains, one heterodimeric inner-arm dynein and six inner-arm dyneins. Each of the heavy chains is reported to have different mechanical properties. They are precisely arranged along doublet microtubules and regulated in a coordinated fashion to produce periodic flagellar beating. To obtain a hint of this complexity, we have carried out in vitro motility assays and compared mechanical properties of various dynein heavy chains, such as velocity of microtubule sliding and processivity. Furthermore, we measured the sliding velocity of microtubules driven by a pairwise mixture of the faster dynein and the slower dynein at various ratios and evaluated the effect of the slower dyneins on the microtubule translocation by the faster ones. We found that the slower dynein would not significantly retard the microtubule translocation by the faster dynein but could be recruited into the translocation of microtubules in the medium velocity. The DNA-origami techniques in which cytoplasmic dyneins were combined together on a DNA-origami rod reveal auto-inhibition between dynein molecules and strain-dependent release from the auto-inhibition. We review these methods for the bottom-up and directed assembly of modular constructs in vitro and we highlight how they shed light on the self-organized coordination of dyneins at force generation in the axoneme.

1176-Symp
Dynein-Mediated Positioning of Microtubule Asters in 3D Confinement
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Important functions of eukaryotic cells such motility and division depend sensitively on cytoskeletal mechanics and organization. In particular, microtubules are stiff dynamic polymers that can generate pushing and pulling forces. To fulfill biological functions, microtubules adopt specific spatial patterns, like the mitotic spindle during cell division. How the shape and size of cells, as well as the presence of pushing and/or pulling forces influence this organization is in many cases still unclear. To assess the influence of confinement on microtubule self-organization, we reconstitute a dynamic microtubule cytoskeleton inside 3D water-in-oil emulsion droplets, using lipids that can optionally be functionalized with active dynein molecular motors. We study the positioning of centrosomes, from which microtubules are nucleated that exert pushing and/or dynein-mediated pulling forces when reaching the boundary. We show that the central position of one centrosome confined in a spherical droplet is drastically destabilized by pushing forces, while pulling forces tend to center the centrosome. Interestingly, when two centrosomes are present, pushing forces cause the centrosomes to find a stable position at opposite sides of the droplet. When both pushing and pulling forces are present, two centrosomes adopt an equilibrium position balancing the centering effect of the dynein-mediated pulling forces with the repulsion effect of the two centrosomes, thereby reproducing a 'mitotic spindle' like organization. These experiments set the stage for a better understanding of the role of additional mitotic spindle components such as mitotic motors and crosslinkers that we plan to add to our system.

1177-Symp
New Methods for Molecular Motor and Cell Motility Research
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Several novel methods for high-speed, high resolution microscopy have enabled new insights into function of molecular motors in vitro and in live cells. Several groups have developed methods to track the position and orientation of bi-functional organic fluorophores or quantum rods bound to specific motor subunits. We have now improved the time resolution by time-multiplexing excitation polarization every 100 μs and resolving structural changes by the consequent sudden changes in photon collection rates. In addition, we developed a multi-channel change-point method to detect structural states within